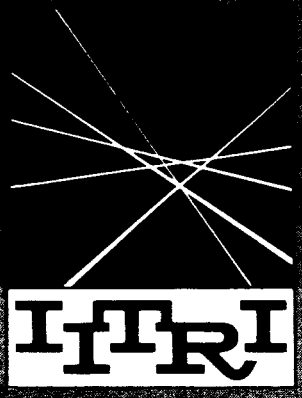

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LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

15 Nov. 1963 - 15 Feb. 1964

National Aeronautics
and Space Administration
Washington, D.C.

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IIT RESEARCH INSTITUTE

Report No. IITRI-C194-12
(Quarterly Status Report)

LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

November 15, 1963, to February 15, 1964

National Aeronautics and Space Administration
(NASA Contract No. NASr-22)
IITRI Project C194

I. INTRODUCTION

The survival of Bacillus subtilis var. globigii in a simulated Martian environment modified by addition of 1% organic medium and moisture concentrations of 0.25, 2.0, 4.9, and 21.7% was studied. The organism survived this environment, but only by virtue of its ability to form spores. B. subtilis spores placed in the simulated Martian environment modified by (a) addition of 10% organic medium plus 8% moisture or (b) addition of 10% organic medium plus 16% moisture plus 5% oxygen did not germinate during the first freeze-thaw cycle, even when they were given prior heat-shock treatment. The decrease observed in total and spore counts after 1 and 2 weeks of exposure could indicate that the spores germinate but do not survive.

A strain of Pseudomonas aeruginosa did not survive in the simulated Martian environment modified by addition of 10% organic medium plus 10% moisture. The death of this organism was very rapid during the first thaw cycle. In subsequent

The fungal component from the lichen Teloschistes chrysophthalmus was isolated by washing the lichen thalli in cold tap water for 30 min and soaking them overnight in sterile distilled water. Fragments were excised from thalli and macerated in a 25-ml Potter-Elvehjem tissue homogenizer containing 10 ml of sterile water and 0.1 ml of Tween 80. The macerated tissue was streaked on the surface of unacidified potato dextrose agar (Difco) and incubated at 20°C for 12 days. Then 1-cm squares of the agar containing the mycobiont were aseptically transferred to mycological agar (Difco) acidified to pH 4.8 and maintained at room temperature.

The diffusible pigments of lichen and mycobiont were extracted from agar with hot 95% ethanol according to the method of Asahina and Shibata.[2]

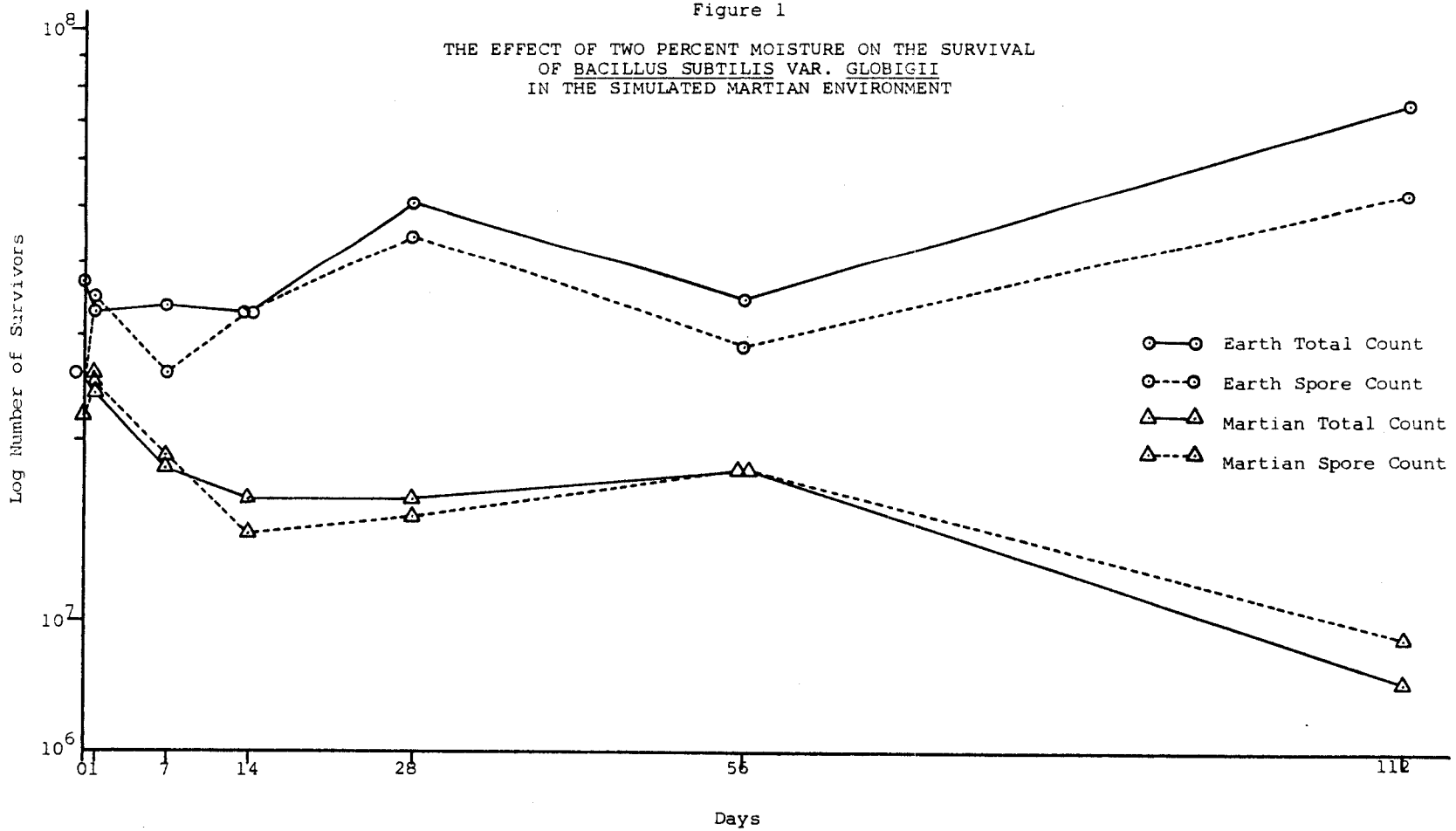
III. RESULTS AND DISCUSSION

The survival of B. subtilis in the simulated Martian environment with 1% organic medium plus 2% moisture is shown in Figure 1. Initially, 70 to 84% of the cells present were spores, and after one freeze-thaw cycle essentially all the remaining cells were spores. The concentrations of organic medium and moisture were not sufficient to support the growth of this organism. The number of viable cells recovered from the tubes

[2]Asahina and Shibata, "Chemistry of Lichen Substances," Tokyo, 1954.

Figure 1

THE EFFECT OF TWO PERCENT MOISTURE ON THE SURVIVAL OF BACILLUS SUBTILIS VAR. GLOBIGII IN THE SIMULATED MARTIAN ENVIRONMENT



of the experimental group decreased slightly over the 112-day exposure period.

Figure 2 shows the effect of higher moisture levels and a 1% organic medium on the survival of B. subtilis. Moisture concentrations of 4.9 and 21.7% had an initial effect on survival. Greater numbers of bacteria survived the flushing and inoculating procedures in the tubes containing the higher moistures. The total count curves show a relationship between the moisture content and the high initial death rate. The slight initial increases in spore counts were perhaps due to the moisture, but the decreasing spore counts after 28 days of exposure indicated the effect was not a lasting one.

Figures 3, 4, and 5 show the effect of freezing and thawing on B. subtilis, Ps. aeruginosa, and B. cereus with 10% organic medium added to the simulated Martian soil. Plate counts were done hourly for the first 3 hr of freezing and first 6 hr of thawing.

Heat-shocked spore suspensions of B. subtilis and B. cereus were used to determine whether these spores are capable of germinating in the simulated Martian environment, and also whether germinated spores multiply and subsequently sporulate. Figure 3 shows the effect of 0.5% oxygen and increased moisture content (16%) on the B. subtilis spore suspension. No effect was noticed on the germination potential of the spores through a freeze-thaw

Figure 2

THE EFFECT OF VARYING CONCENTRATIONS OF MOISTURE ON THE SURVIVAL OF BACILLUS SUBTILIS VAR. GLOBIGII IN THE SIMULATED MARTIAN ENVIRONMENT

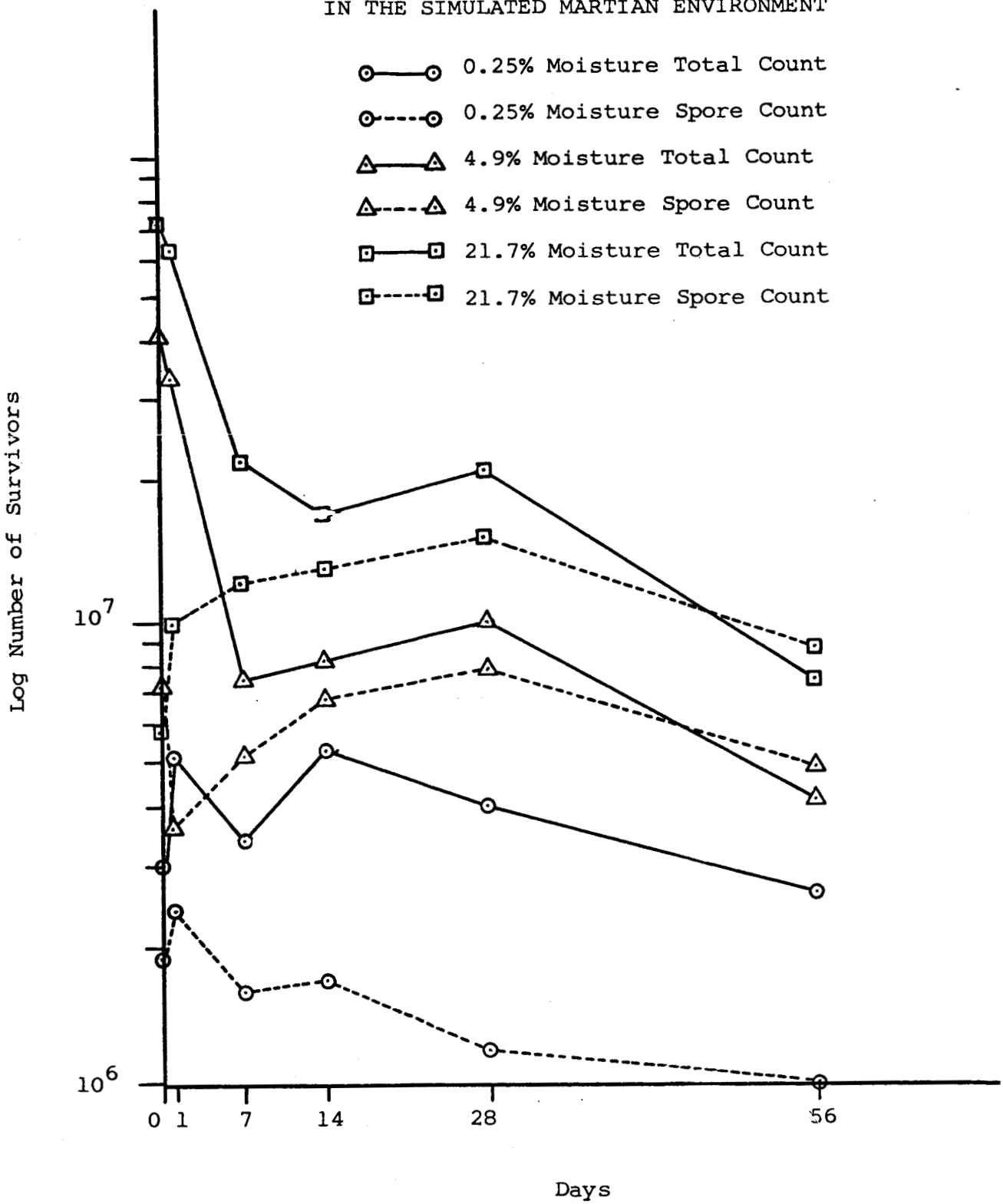
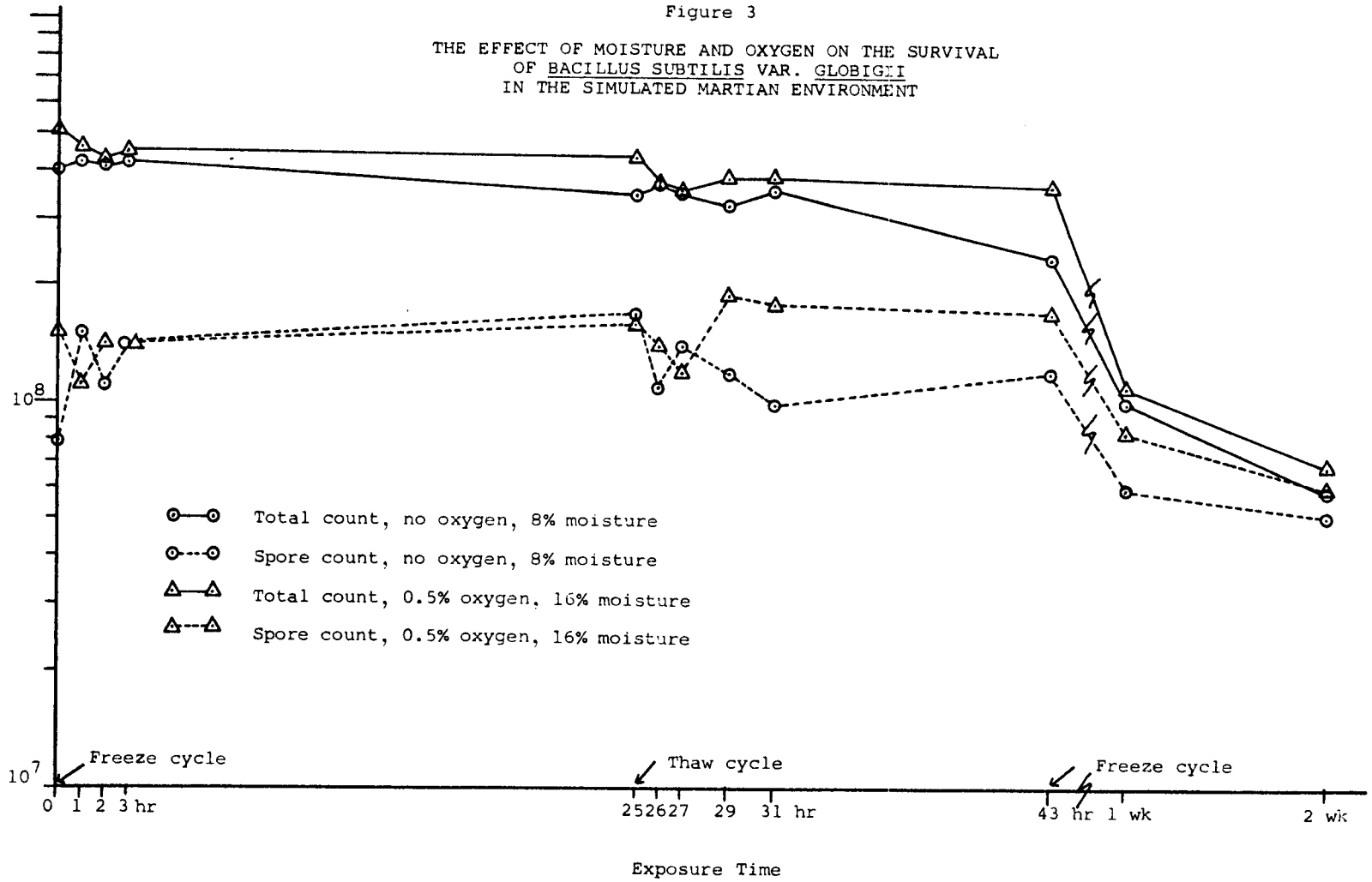


Figure 3

THE EFFECT OF MOISTURE AND OXYGEN ON THE SURVIVAL OF BACILLUS SUBTILIS VAR. GLOBIGII IN THE SIMULATED MARTIAN ENVIRONMENT



cycle. The decrease in total and spore counts after 1 and 2 weeks of exposure could indicate that the spores are germinating but not surviving. This experiment will be continued for 4 months.

The survival of Ps. aeruginosa in the simulated Martian environment with 10% moisture is shown in Figure 4. The organisms survived the initial freezing with no significant change in the number of viable cells; but a very rapid decrease began 2 hr after thawing was initiated, and 1.4% of the cells were recovered at the end of 18 hr. After a 1-week exposure both aerobic and anaerobic cell counts had decreased to less than 0.02% of the initial count. Qualitative tests for oxidase, cytochrome C, and nitrate and nitrite reduction did not show any differences between aerobically and anaerobically grown cells.

The effect of the simulated Martian environment with 20% moisture on B. cereus spores is shown in Figure 5. The concentrations of organic medium and moisture had no effect on the germination of spores during the initial freeze-thaw cycle or after subsequent cycles. Survival of this organism will be examined over a 4-month exposure period.

Several unsuccessful attempts were made to isolate the lichenized fungus by the spore method of Hale[3] and Ahmadjian.[4]

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[3]Hale, Bull. Torrey Bot. Club, 82, 182-187, 1958.
[4]Ahmadjian, Bryologist, 64, 168-179, 1961.

Figure 4

THE EFFECT OF TEN PERCENT MOISTURE ON THE SURVIVAL
OF PSEUDOMONAS AERUGINOSA IN THE SIMULATED MARTIAN ENVIRONMENT

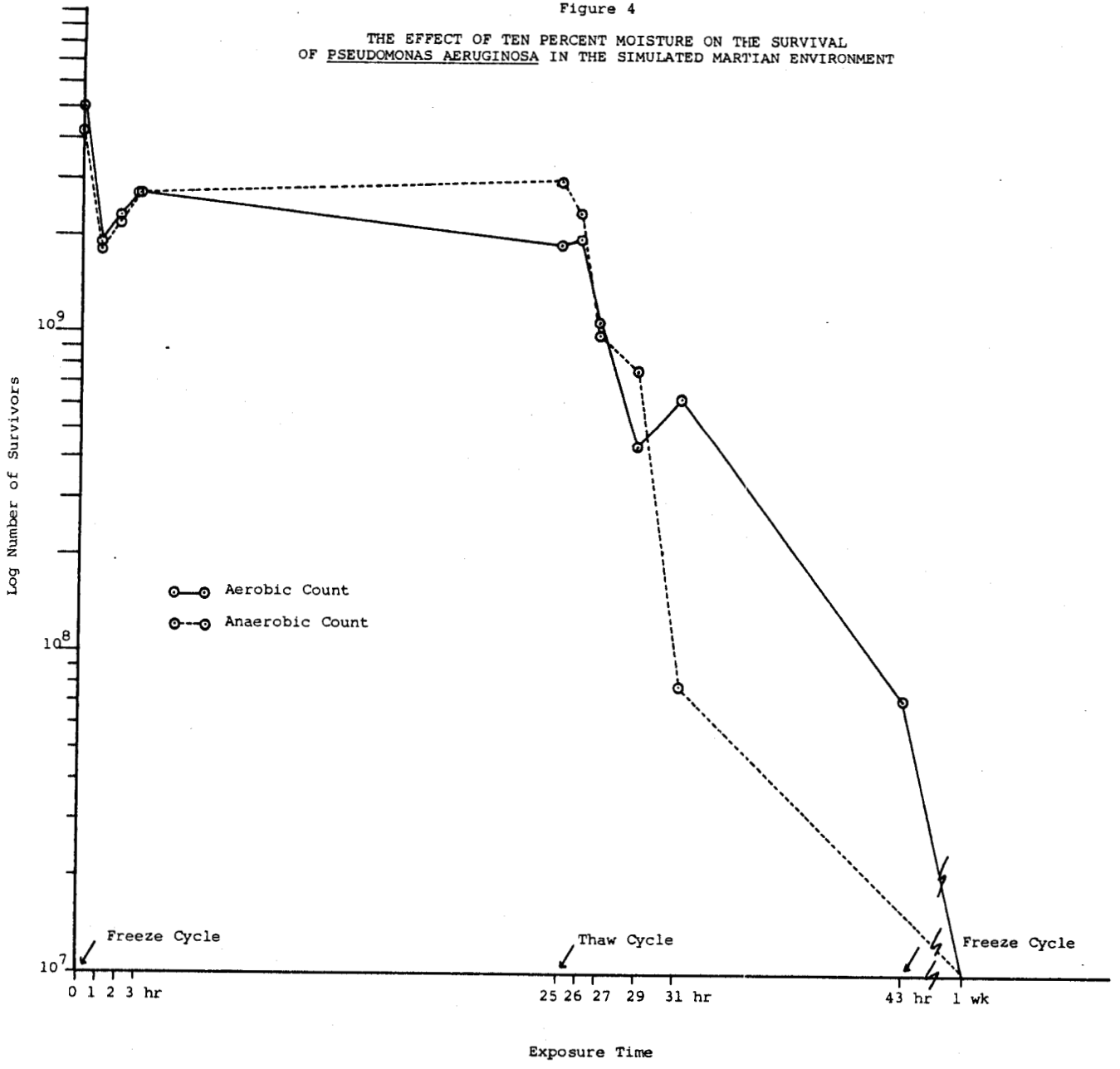
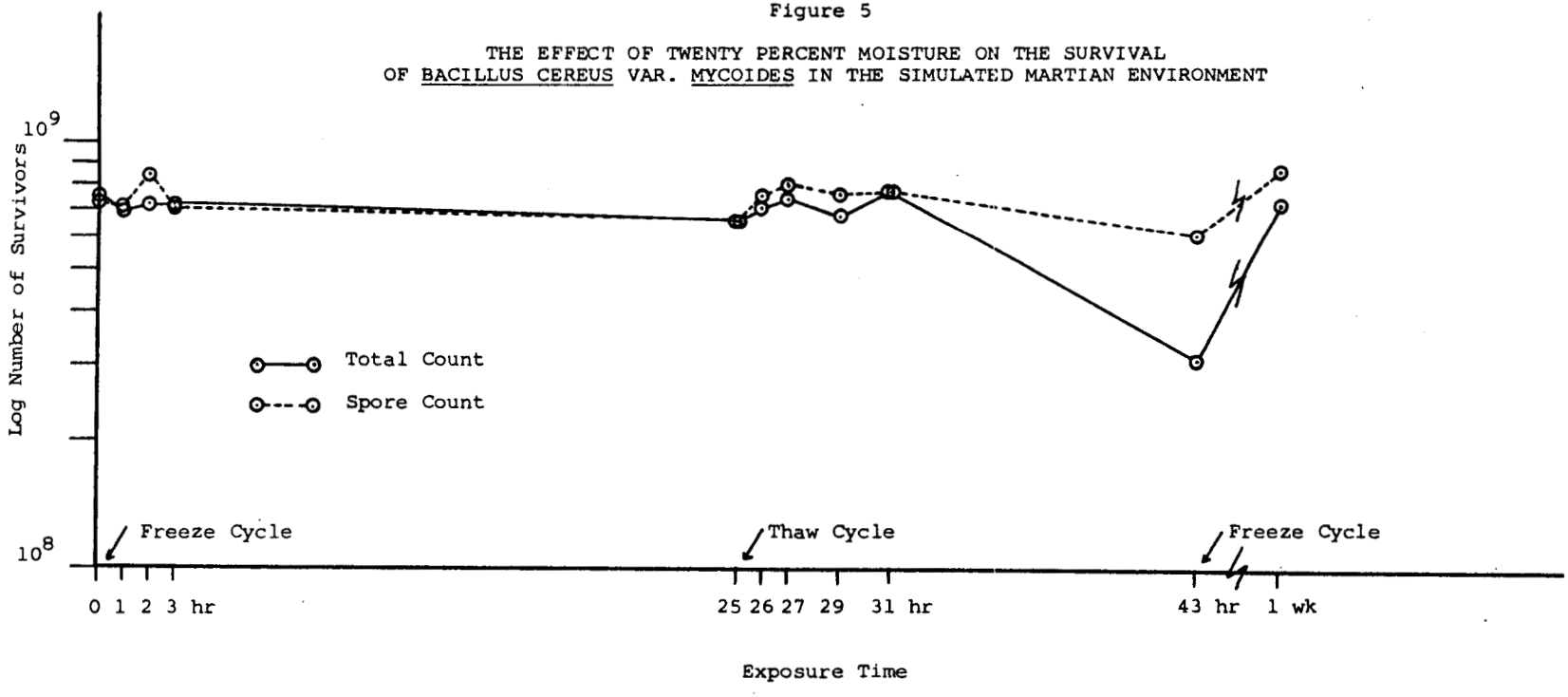


Figure 5

THE EFFECT OF TWENTY PERCENT MOISTURE ON THE SURVIVAL
OF BACILLUS CEREUS VAR. MYCOIDES IN THE SIMULATED MARTIAN ENVIRONMENT



Successful isolation was achieved by macerating thalli and streaking them on unacidified potato dextrose agar. Hyphal fragments germinated in 12 hr. Aspergillus niger, Penicillium spp., and Alternaria spp. were observed as contaminants. Twelve days after inoculation the mycobiont produced a yellow, water-soluble pigment whose margin in the agar coincided with a distinct zone of inhibition to the contaminants.

Small squares of agar containing the mycobiont were transferred to acidified mycological agar. In pure culture the colonies of the fungus changed from white to yellowish brown within 3 weeks. The fungus produced a large amount of pigment, which was observed as droplets on top of the mycelial pad.

Since it has not been conclusively demonstrated that lichenized fungi produce reproductive or other taxonomically significant structures, the mycobiont was identified by comparison of the pigment it produced with that produced by the parent lichen.

Absorption spectra of pigments extracted from the parent lichen and the mycobiont exhibited an absorption maximum at 430 mμ in the visible range. The absorption in the ultraviolet range was very great; the resolution of the curve is being determined. The pigment extracted from the parent lichen was temperature sensitive and turned clear at room temperature but remained yellow at 15°C.

IV. SUMMARY

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Incorporation of 4.9 or 21.7% moisture into the dry simulated Martian soil modified by the addition of 1% organic medium increased the number of B. subtilis surviving the inoculating and flushing procedures. Lower moisture concentrations, 2.0 and 0.25%, did not have this effect. However, the death rate was greater in the tubes with 4.9 and 21.7% moisture. Thus, after 56 days of exposure there was no significant difference between the groups.

B. subtilis spores in Martian environment modified by 10% organic medium appeared to be slightly affected by (a) 8% moisture or by (b) 16% moisture plus 0.5% oxygen. The decrease in total and spore counts indicates that the spores germinate but do not survive.

Less than 0.02% of Ps. aeruginosa cells survived a 1-week exposure to Martian environment modified by 10% organic medium and 10% moisture.

B. cereus spores survived the simulated Martian environment modified by 10% organic medium plus 20% moisture, but there was no apparent germination.

Isolation of the fungal component from the lichen T. chrysophthalmus was accomplished. A method for identifying the mycobiont by absorption spectra of diffusible pigments and lichenolic acids has been initiated.

AUTHOR

V. FUTURE PLANS

A critical examination of the function of moisture on the growth of bacteria during constant and diurnal temperatures has been initiated in order to establish the minimum environmental conditions necessary for growth. These studies will be expanded to include diurnal temperatures of various durations and soils containing different concentrations of moisture plus organic medium.

Methods will be investigated to study the effects of moisture, organic medium, and temperature on the germination of spores in the simulated Martian environment.

Similar studies will be conducted with T. chrysophthalmus to define the growth-limiting factors inherent to the simulated Martian environment.

The effect of ultraviolet light on lichen growth and physiology and on the ability of lichens to imbibe substantial quantities of water from ambient atmospheres will be investigated.

The program will be greatly stimulated by the acquisition of soil and lichen specimens from Antarctica. These specimens are being collected by Dr. Rudolph of the Ohio State University's Polar Institute.

VI. RECORDS AND PERSONNEL

The experimental data are recorded in Logbooks C 13795, C 14081, and C14419.

Technical assistance was given by Miss Charlene Berger.

Respectfully submitted,

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